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A nationwide survey on seroprevalence of *Neospora caninum* infection in beef cattle in Uruguay

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Abstract

Bovine abortions due to *Neospora caninum* infection have been reported worldwide and its economic impact on the beef industry has been acknowledged as a problem. Uruguay has the largest export value of beef per acre in South America. However, no data on the prevalence of *N. caninum* infection have been available in this country. The objective of this study was to estimate the prevalence and distribution of *N. caninum* infection in beef cattle in Uruguay through a nationwide survey. A two stage sampling design was used with farms being selected in stage one and animals being selected in stage two. A brief questionnaire was administered on each farm. Seroprevalence of *N. caninum* in 4444 beef cattle from 229 farms in all the counties, except Montevideo, of Uruguay was determined by an ELISA. The data were then analyzed to identify associations between infection and variables such as type of animal (cow or heifer), herd size, use of veterinary advice, productivity of the soil in relation to the national average, use of improved grass, use of mineral salts, use of supplemental feed, and presence of a dog(s) on the farm. The estimated proportion of positive farms for all the beef cattle operations was 69.2% (95% confidence interval [CI], 53.7–84.7). The overall cattle seroprevalence was estimated as 13.9% (95% CI, 11.6–16.3). The prevalence estimation by animal category was 14.3% (95% CI, 11.4–17.2) for beef cows and 12.9% (95% CI, 10.0–15.8) for beef heifers. There was no significant difference in the estimated prevalence between the two animal types. There was no significant difference in the animal level prevalence of *N. caninum* infection among different herd sizes. None of the herd demographic or management variables was significantly associated with the seropositivity to *N. caninum* infection. In conclusion, these results show that *N. caninum* infection is common among beef herds across Uruguay. Since the beef industry is one of the key industries in Uruguay, the economic effect and risk factors of *N. caninum* infection among beef cattle in this country should be further evaluated in the near future.

Keywords: *Neospora caninum*; ELISA; Uruguay; Seroprevalence

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1. Introduction

In 1989, Thilsted and Dubey (1989) described the *Neospora caninum* as an agent that causes bovine abortion in the USA. Subsequently, bovine abortions due to *N. caninum* infection have been reported worldwide and its economic impact on the cattle industry has been acknowledged as a problem (Anderson et al., 1991; Dubey, 1999).

N. caninum infection in cattle has also been reported in other parts of South America including Argentina, Brazil, and Paraguay (Venturini et al., 1999; Gondim et al., 1999; Osawa et al., 2002; Moore, 2005). The first demonstration of exposure to the organism in Uruguay was in 1997, when 20% of 414 dogs living on farms were positive to the indirect fluorescent antibody test (IFAT) for *N. caninum* (Barber et al., 1997). The first diagnosis of neosporosis in dogs and cattle in Uruguay was by immunohistochemistry and serological techniques in 1999, and neosporosis has been diagnosed routinely since then (Bañales et al., 1999, 2000). In 1999 and 2000, 37% of aborted bovine fetuses submitted to the National Veterinary Reference Laboratory (DILAVE) of Uruguay were confirmed with *N. caninum* (Easton et al., 2001). Kashiwazaki et al. (2004) recently reported seroepidemiology of *N. caninum* infection in a dairy herd in Uruguay. However, no information has been available on the distribution of *N. caninum* infections in beef cattle in this country. The objective of this study was to determine the prevalence and distribution of *N. caninum* infection in beef cattle in Uruguay.

2. Materials and methods

2.1. Area

Uruguay is located between Argentina and Brazil in South America and at latitude between 30° and 35° south with mean daily temperature varying from 13 °C (winter) to 25 °C (summer). It has 176,215 km^2 of land and 80% of its land surface area is occupied by natural and regenerated pastures that are suitable for livestock farming (García-Préchac et al., 2004). Uruguay has a human population of about 3 million and a cattle population of more than 12 million (FAOSTAT Data, 2004). Uruguay has the largest export value of beef per acre in South America. Beef is the largest export in the country and accounts for more than 25% of the total export in dollars (FAO, 2004).

2.2. Survey

To determine the geographic distribution of beef farms in Uruguay, a cross-sectional study of properties and cattle was conducted at a national level. The sampling frame consisted of all farms rearing beef cattle for reproduction which are required to register with the Directorate of Livestock Control (División Contralor de Semovientes: DI.CO.SE) annually. A two stage sampling design was used with farms being selected in stage one and animals being selected in stage two.

In stage one, the farms were randomly selected with probability proportional to the herd size (population of cows and heifers) (Korn and Graubard, 1999). In other words, the probability that a farm is selected was based on the number of animals. The sample size of farms for the questionnaire was 230. This number was calculated to estimate the proportion of the affected farms with a confidence limit of 95% and a precision level of 5%, based on the expected proportion of affected farms of 18%. In stage two, the animals were selected in two categories: cows and heifers. The selection of the animals on each farm was systematic and the sample size was 10 animals in each category (blood samples were collected from around 10 cows and 10 heifers of each farm). This sample size ($n = 10$) was chosen based on a desire to be 95% confident of detecting at least one infected animal if the within-herd prevalence was at least 26% in the group.

When each operation was contacted to participate in the survey a brief questionnaire was administered prior to the collection of blood samples. Producers responded to questions about size of the herd, nutritional management, use of veterinary services, and types of animals present on the farm.

A total of 4444 (2245 cows and 2199 heifers) blood samples from 229 beef herds (farms) located in all the counties of Uruguay, except Montevideo, that is 18 counties, were used for this study. The age of the animals varied from 1 to 3 years old for the heifers and 3 to 10 years old for the cows. Samples were collected from March 2000 to March 2001. Blood without anticoagulant was taken from the jugular or caudal veins and transported to either the central or regional laboratory in individual tubes marked with the identification number of the premise and animal type. There, they were centrifuged and the serum obtained was labeled and stored at $-20\ ^{\circ}C$ until processing. The samples arrived to the laboratory within 48 h after extraction. Data were collected on demographic information for the selected herd. Information on abortion history in these animals was not available.

2.3. ELISA

Between May and September 2001, serum samples were tested for the presence of IgG antibodies to *N. caninum* by an ELISA (Osawa et al., 1998) using water-soluble fraction of sonicated tachyzoites of NC-1 isolate (Dubey et al., 1988), as antigen. Control and sample sera were tested in duplicate. The cut-off OD value was determined as 0.45 because this level was equivalent to a titer of 1:200 in indirect fluorescent antibody test that was considered indicative of *N. caninum* exposure (Kashiwazaki et al., 2004). Serum samples with an OD greater than 0.45 were regarded as positive for anti-*Neospora* antibody.

2.4. Statistical analysis

Seroprevalence in cows and heifers was determined at farm level as well as the county level. The data were analyzed to identify associations between infection and variables such as type of animal (cow or heifer), herd size (less than 300 head, 300–1000 head, or more than 1000 head), veterinary advice (none, on demand, or permanent), productivity of the soil in relation to the national average (less than 76%, between 76 and 90%, between 91 and 110%, between 111 and 130%, or more than 130%), use of improved grass or natural grass, use of mineral salts (yes or no), use of supplemental feed (yes or no), and presence of a dog(s) (yes or no). The infection status of herds and each of the potential risk variables were tested for independence based on the usual Pearson χ^2 for two way tables. To account for the survey design, the statistic was turned into F statistics using a second-order Rao and Scott correction and the p-value for the design-based test (F) was interpreted in the same way as a p-value for the Pearson χ^2 for ‘‘ordinary’’ data. (StataCorp., 2005). For these tests the survey routines of the statistical software, Intercooled STATA version 7 (StataCorp. LP, TX, USA) were used, according to the method previously reported (Dargatz and Hill, 1996). Herds were considered positive if one or more serum samples were positive on the ELISA test. Herd level prevalence was calculated by accounting for the sampling design. Specifically the data were weighted using the inverse of the overall sampling fraction for the individual herd. Animal level prevalence estimates accounted for both the sampling design for selecting the herd as well as the sampling fraction of animals within the herd. Confidence intervals of the estimates and all statistical comparisons accounted for the study design (StataCorp., 2005). The prevalence estimates reported were not adjusted for the sensitivity or specificity of the test.

3. Results

Of the 229 farms tested by the ELISA, 169 farms (73.8%) had seropositive animals. Positive farms were present in 16 of the 18 counties studied (Table 1). Of the 4444 Uruguayan cattle tested by the ELISA, 589 animals (13.25%) were seropositive. Sample prevalence in the cows was 13.6% (306/2245) and that in the heifers was 12.9% (283/2199). The OD values for cows with positive test results ranged from 0.451 to 2.095 with a mean value of 0.578. For heifers with a positive test the OD values ranged from 0.452 to 2.609 with a mean value of 0.664. Overall, 50.9% (86/169) of positive farms had equal to or less than 15% positive animals and 75.7% (128/169) of positive farms had equal to or less than 25% positive animals (Table 2).

Population estimates for beef cattle herds in Uruguay were adjusted for the study design. Overall, 69.2% (95% confidence interval [CI], 53.7–84.7) of herds with one or more beef cattle had evidence of exposure to *N. caninum* in Uruguay. At the animal level, the overall cattle prevalence was estimated as 13.9% (95% CI, 11.6–16.3). The estimated prevalence by animal category was 14.3% (95% CI, 11.4–17.2) for beef cows and 12.9% (95% CI, 10.0–15.8) for beef heifers. There was no significant difference in the estimated prevalence between the two animal types ($p = 0.49$). There was no significant difference in the animal level prevalence of *N. caninum* infection among different herd sizes.

None of the null hypothesis of independence between the management variables and the seropositivity to *N. caninum* infection were rejected i.e. there

Table 1
Frequency of serum with anti-*Neospora caninum* antibodies at herd and animal levels in 18 counties of Uruguay

Counties	Herd farm(+)/n_f	Cow cow(+)/n_c	Heifer heifer(+)/n_h	Frequency in total (%)
Artigas	6/9 (66.7)	12/88 (13.6)	3/90 (3)	8.4
Canelones	0/2 (0.0)	0/20 (0.0)	0/10 (0.0)	0
Cerro Largo	15/17 (88.2)	19/164 (11.6)	38/165 (23.0)	17.3
Colonia	0/3 (0.0)	0/30 (0.0)	0/30 (0.0)	0
Durazno	11/13 (84.6)	19/129 (14.7)	23/128 (18.0)	16.3
Flores	6/11 (54.5)	3/110 (2.7)	5/110 (4.5)	3.6
Florída	8/10 (80.0)	15/99 (15.2)	13/100 (13.0)	14.1
Lavalleja	11/15 (73.3)	11/147 (7.5)	23/143 (16.1)	11.7
Maldonado	1/1 (100.0)	1/10 (10.0)	0/10 (0.0)	5.0
Paysandú	9/14 (64.3)	15/134 (11.2)	19/139 (13.7)	12.5
Rio Negro	12/18 (66.7)	17/178 (9.6)	4/173 (2.3)	6.0
Rivera	15/21 (71.4)	33/204 (16.2)	32/188 (17.0)	16.6
Rocha	16/19 (84.2)	54/187 (28.9)	33/189 (17.5)	23.1
Salto	22/29 (75.9)	42/284 (14.8)	37/288 (12.8)	13.8
San José	2/2 (100.0)	1/20 (5.0)	6/19 (31.6)	17.9
Soriano	16/20 (80.0)	28/199 (14.1)	34/194 (17.5)	15.8
Tacuarembó	12/15 (80.0)	27/150 (18.0)	9/149 (6.0)	12.0
Treinta y Tres	7/10 (70.0)	9/92 (9.8)	4/74 (5.4)	7.8
Total	169/229 (73.8)	306/2245 (13.6)	283/2199 (12.9)	13.3

Farm(+): number of farms with at least one serum sample with anti-*N. caninum* antibodies; cow(+): number of cows with anti-*N. caninum* antibodies; heifer(+): number of heifers with anti-*N. caninum* antibodies; n_f: number of herds sampled; n_c: number of cows sampled; n_h: number of heifers sampled.

was no significant association with serologic status and herd demographic or management factors evaluated (Table 3).

4. Discussion

The overall estimated animal level seroprevalence of *N. caninum* infection for beef cattle (cows and heifers)

Table 2
Distribution of the within-herd raw prevalence of anti-*N. caninum* antibodies

% of positive animals[a]	No. of herds (%)
0	60 (26.2)
>0, ≤15	86 (37.6)
>15, ≤25	42 (18.3)
>25, ≤50	37 (16.2)
>50, ≤80	3 (1.3)
>80	1 (0.4)
Total	229 (100)

[a] Approximately 20 animals per herd sampled.

Table 3
Relationship between anti-*Neospora caninum* antibodies on farms and potential risk factors

Variable	Categories	Design-based test[a]	*p*-Value
Herd size[b]	3	0.30	0.63
Veterinary advice[c]	3	0.07	0.86
Productivity of the soil[d]	5	1.10	0.35
Improved grass[e]	2	0.04	0.84
Salts[f]	2	0.77	0.44
Supplemental feed[g]	2	0.74	0.39
Presence of dog(s)[h]	2	0.18	0.67

[a] Design-based test for independence appropriate for clustered data based on Pearson χ^2 for two way tables.
[b] Less than 300 head, 300–1000, or more than 1000.
[c] No, on demand, or permanent.
[d] Productivity in relation to average country soil agriculture production (less than 76%, between 76 and 90%, between 91 and 110%, between 111 and 130%, or more than 130%).
[e] Use of improved grass or natural grass.
[f] Use of mineral salts or not.
[g] Use of supplemental feed or not.
[h] Presence of at least one dog or no dogs on farm.

was 13.9% (95% CI, 11.6–16.3). By comparison the proportion of seropositive beef cattle in Argentina was 4.7% (Moore et al., 2002) and in Paraguay was 29.8% (Osawa et al., 2002). Although herd exposure to the agent was relatively high, 69.2% (95% confidence interval, 53.7–84.7), this figure could underestimate the true prevalence because we only sampled around 20 animals per farm, farms with low within-herd prevalence may have been misclassified as negative. This potential misclassification of herd status could also affect the analysis of potential risk factors.

The parasite appears to be widely distributed geographically with nearly all (16 of 18) counties where cattle were sampled having positive herds. These results agree with data from diagnostic investigations of bovine abortions performed by the National Veterinary Reference Laboratory for Uruguay for 1999 and 2001 (Easton et al., 2001). Kashiwazaki et al. (2004) reported a prevalence of 61.3% in 155 cows, 31 heifers, and 31 calves that were randomly bred from a small dairy herd where there was abortion problem. The national prevalence among beef cattle in our study was lower than that figure, and the percentage of the herds having high animal level prevalence was low. These results could be due to a number of factors including an overall higher exposure among animals in dairy herds or that this particular herd reported by Kashiwazaki et al. (2004) was having on-going abortions due to *N. caninum* (Osawa et al., 2002; Moore, 2005).

In this study we examined the distribution of *N. caninum* infection in beef herds in Uruguay and estimated that 14.3 and 12.9% of beef cows and heifers, respectively, have been exposed to this coccidian parasite. From the results on the prevalence in cows and heifers in this country, it is suggested that the major transmission route may be transplacental. If horizontal transmission was an important route of *N. caninum* infection, seroprevalence in cows would have been much higher than that in heifers because the chance of being exposed to the parasite becomes higher as the animal gets older. Furthermore, the reason why the major route of *N. caninum* infection in Uruguay may be vertical transmission can be explained by the results showing that there was no difference in seroprevalence between beef herds that were given silage and/or mixed feed other than grass and those given only grass, and there was no clear-cut association between seroprevalence and presence of dogs in beef herds. Nonetheless, the possibility of the horizontal transmission occurring among the beef cattle in Uruguay cannot be denied. Silage or mixed feed may have been contaminated with *N. caninum* oocysts (McAllister et al., 1998). No decisive conclusion could be drawn on the involvement of the canine species in the transmission of the bovine infection in Uruguay, since no samples from the dogs in the targeted farms were available for anti-*Neospora* antibodies in this study. More study is required to clarify the transmission of *N. caninum* infection among Uruguayan cattle.

The seroprevalence we found in this study is of help to delve deeper into neosporosis that has been recognized as a cause of abortions in beef cattle in Uruguay (Bañales et al., 1999), and to assess its economic impact and risk factors that could be implicated in *N. caninum* infection or abortion in the future. Studies in the United States have suggested a significant economic cost to producers due to losses associated with *N. caninum* infections (Thurmond and Hietala, 1996). It is unclear if the impacts will be the same under the production conditions in Uruguay. Further investigation of the economic and production impacts of *N. caninum* in beef and dairy herds in Uruguay is warranted.

In conclusion, *N. caninum* infection is common among beef herds across Uruguay. Since the beef industry is one of the key industries in Uruguay, the economic effect and risk factors of *N. caninum* infection among beef cattle in this country should be estimated in the near future.

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